pairs are considered: (1) a Noble-Noble cell pair that represents adjacent cells in Purkinje network, (2) a pair of DiFranceso-Noble cells that represents adjacent SA nodal cells, and (3) a model of Noble cell coupled to Luo-Rudy cell model, which represents an interacting pair of a Purkinje fiber and a ventricular myocyte. Bistability is demonstrated in all the three cases and this bistability might be an underlying factor behind cardiac memory. Focused analysis of a pair of Noble cell models showed that bistability is obtained only when the properties of GJs ''match'' with the properties of the pair of cells that is coupled by the GJs. This study showed the role of GJ channels in cardiac memory.

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Suppressing Early Afterdepolarizations in Cardiac Myocytes by Shaping a Modified Ca^{2+} Conductance Under Dynamic Clamp

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Early afterdepolarizations (EADs) are highly arrhythmogenic alterations of the cardiac action potential (AP) characterized as transient reversals of repolarization during AP phase 2 or 3. Using the dynamic clamp technique, we combined mathematical modeling and electrophysiology in real-time to introduce a virtual L-type Ca current ($I_{Ca,L}$) with tunable biophysical properties in dissociated rabbit ventricular myocytes under current clamp. We sought to **i)** understand the etiology of EAD development and their dependence on $I_{Ca,L}$ and **ii)** identify therapeutic strategies to suppress EADs by shaping a new L-type current. The native $I_{Ca,L}$ was blocked with 20μM nifedipine and replaced by a ''virtual'' $I_{Ca,L}$. A modest 4mV leftward shift in the $V_{1/2}$ of activation prolonged APD_{90} to 441 ± 100ms inducing EADs in 25% of APs, while a 5mV leftward shift further prolonged APD_{90} to 1260 ± 129ms, inducing EADs in 100% of APs thus revealing a striking dependence of EAD frequency on the $I_{Ca,L}$ activation $V_{1/2}$. To investigate the therapeutic potential of $I_{Ca,L}$ modifications in suppressing EADs, we first induced a robust EAD regime by exposing cardiomyocytes either to hypokalemia (2.4 mM KCl) or oxidative stress (0.6mM H_2O_2, PCL=5s). EADs were completely abolished with 20μM nifedipine but promptly restored under dynamic clamp by a virtual Ca^{2+} current modeled as modified by H_2O_2. However, a 5mV rightward shift in the $I_{Ca,L}$ $V_{1/2}$ of activation dramatically reduced APD and EAD frequency from 100% to <5%. Shifting the $V_{1/2}$ of the steady-state inactivation curve by 7mV towards more negative potentials completely abolished EADs. Both maneuvers reduced the $I_{Ca,L}$ window current region and abolished EADs, while the predicted Ca transients were largely unaffected. These results support the hypothesis that EAD occurrence can be controlled by fine-tuning the biophysical properties of the L-type window current.

2373-Pos Board B359

The pH Sensitivity of the Cardiac Na^+/Ca^{2+} Exchanger Depend on Calcium Binding Domains (CBD) of NCX1

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The major pathway of Ca^{2+} extrusion from cardiomyocytes is the cardiac Na^+/Ca^{2+} exchanger (NCX1) and it thus plays a critical role in regulating intracellular calcium $[Ca^{2+}]_{i,}$. We have investigated how intracellular pH (pH_i) regulates NCX1 activity in intact cardiomyocytes. The NCX1 current (I_{NCX}) was used to measure NCX1 turnover rate in patch clamped intact rat ventricular myocytes. The dependence of I_{NCX} on $[Ca^{2+}]_i$ was determined at different levels of pH_i (7.2 to 6.9) using an ammonium chloride "rebound" method while extracellular pH (7.4) was kept constant. At pH_i = 7.2 and $[Ca^{2+}]_i$ < 120 nmol/L, I_{NCX} was less than 4% of the maximally Ca^{2+}-activated value. At pH_i = 7.2 I_{NCX} increased steeply as $[Ca^{2+}]_i$ increased (apparent threshold at $[Ca^{2+}]_i$ between 130 - 150 nmol/L, with a Hill coefficient (n_H) of 8.0 ± 0.7 and $K_{0.5}$ = 310 ± 5 nmol/L). At pH_i = 6.87, the threshold of Ca^{2+}-dependent activation of I_{NCX} was shifted to much higher $[Ca^{2+}]_i$ (600-700 nmol/L) and the relationship was similarly steep (n_H = 8.0 ± 0.8) with $K_{0.5}$ = 1042 ± 15 nmol/L. The V_{max} of Ca^{2+}-dependent activation of I_{NCX} was not significantly altered by low pH_i. When decreasing pH from 7.2 to 6.9, the $^{45}Ca^{2+}$ binding affinity to the two calcium binding domains (CBD) of isolated protein regions was measured. The K_d values were measured at pH 7.2 for CBD1 (K_d = 0.39 ± 0.06 mol/L) and CBD2 (K_d=18.4 ± 6 mol/L) and each decreased 2-3 fold at pH 6.9. We conclude that NCX1 can be "switched off" by intracellular acidification and that this is due to the competitive binding of protons to the two regulatory calcium binding domains of NCX, CBD1 and CBD2.

2374-Pos Board B360

NADH as an Endogenous Marker of Cardiac Tissue Injury at the Site of Radiofrequency Ablation

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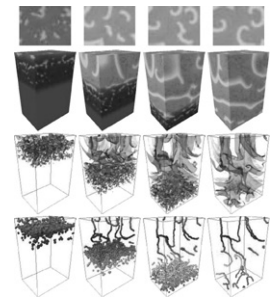
Atrial fibrillation (AF) remains the most commonly occurring cardiac arrhythmia; its incidence is associated with a lower quality of life including a higher rate of morbidity & mortality. Percutaneous left atrial catheter ablation for the purpose of eliminating AF has become a common treatment option and is currently being investigated for superiority over medical therapy in a large clinical trial. RFA produces lesions that can block the spread of electrical activity from the sites of abnormal activity including pulmonary veins. Today, there are limited means for real time monitoring of tissue injury during the RFA procedure. To address this need, we explored the fluorescence of endogenous NADH as a possible live marker of tissue injury during the ablation procedure. Studies were conducted in blood-free and blood-perfused isolated rat hearts. Epicardial RFA lesions were seen as areas of low NADH fluorescence which encompassed both irreversible and reversibly damaged tissue. Their size significantly exceeded TTC negative staining (TTC - Triphenyl Tetrazolium Chloride, a redox indicator used to differentiate between metabolically active and inactive tissues). Real-time monitoring of NADH fluorescence allowed visualization of gaps of viable tissue between the RFA lesions. Dual recordings of NADH and epicardial electrical activity linked these gaps to the occurrence of post-ablation reentries. We conclude that fluorescence of endogenous NADH can assist visualization of injured epicardial tissue near the tip of the RFA catheter lesions.

2375-Pos Board B361

Ischemic Boundary as a Source of Scroll Waves: A Three-Dimensional View

Vadim Biktashev, Ara Arutunyan, **Narine Sarvazyan**.



This study focuses on abnormal waves of activity formed within the border zone of an ischemic region. We considered three-dimensional block of cardiac tissue in which a steep gradient in cell-to-cell coupling constituted border-like conditions. This border was then placed under time-dependent conditions that promote cell automaticity and moved in space. The events mimicked complex clinical conditions associated with ischemia-reperfusion. Network behaviour was modeled based on Beeler-Reuter formalism of cardiac cell taking into account individual cell heterogeneity. The observed events ranged from single cell activity, to spherically spreading sources and multiple scroll waves. A bell-shaped relationship between the speed at which the coupling gradient moves in space and the probability of a scroll wave to escape was established. The data provide insights into possible mechanisms of ectopic activity formation and its escape from the boundary of ischemic tissue.

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Biophysical Characterization of I_{ks} Channels in Cardiomyocytes Derived from Human Embryonic Stem Cells Reveals Insights Into Stoichiometry of KCNQ1-KCNE1 Complex

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The slowly activating I_{Ks} cardiac potassium channels are heteromultimers composed of four identical α-subunits (KCNQ1) which assemble with auxiliary -subunits (KCNE1) that confer distinct biophysical properties on the channels: a slowing of activation and deactivation kinetics and a large depolarizing shift in activation. Here we characterize the electrophysiological properties of endogenous I_{Ks} channels in single cardiomyocytes derived from human embryonic stem cells (hESC-CMs). I_{Ks} was identified as Chromanol 293B-sensitive current measured during prolonged depolarization. The mid-point of I_{Ks} activation occurred at 9.1 ± 2.4 mV (n=11), between that of KCNQ1 alone ($V_{1/2}$= -21.3 ± 1.5 mV, n=4) and KCNQ1 expressed with a saturating amount of KCNE1 ($V_{1/2}$=27.2 ± 1.2 mV, n=5) in HEK293 cells. To determine whether ESC-CM background affects I_{Ks} biophysical properties, we engineered KCNQ1 channels to impart a sensitivity to charybdotoxin (CTX) and transiently expressed these channels (CTX-KCNQ1) in hESC-CMs with or without KCNE1. We used CTX to dissect the recombinant I_{Ks} channels from endogenous channels and found they shared similar properties as in HEK293 cells (CTX-KCNQ1: $V_{1/2}$=-27.2 ± 4.4 mV, n=4; CTX-KCNQ1 + KCNE1: $V_{1/2}$=30.9 ± 1.1 mV, n=5). This confirms that the mid-point of activation of endogenous hESC-CM I_{Ks} lies between KCNQ1 alone and KCNQ1/KCNE1 heteromultimers, suggesting that endogenous I_{Ks} is produced by KCNQ1 channels that are not saturated by KCNE1. We tested this hypothesis by overexpressing KCNE1 in hESC-CMs and found that endogenous I_{Ks} channels activated more slowly and at more positive voltages ($V_{1/2}$=34.2 ± 1.4 mV, n=4) than in non-transfected cells. This is the first comprehensive report of the biophysical properties of I_{Ks} in hESC-CMs, suggesting that endogenous I_{Ks} is produced by KCNQ1 channels that are assembling, but not saturated with, KCNE1 subunits.